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Stability, nutritional and sensory characteristics of French salad dressing made with mannoprotein from spent brewer's yeast

Adma Nadja Ferreira de Melo [a], Evandro Leite de Souza [b], Vilma Barbosa da Silva Araujo [a], Marciane Magnani [a, *]

[a] Laboratory of Biochemistry of Foods, Department of Food Engineering, Federal University of Paraíba, João Pessoa, Brazil
[b] Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba, João Pessoa, Brazil

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ABSTRACT

The effects of refrigerated storage over 28 days on the stability, nutritional and sensory characteristics of French salad dressing made with either mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) (MP) (X1), MP and soy lecithin (SL) (X2) or with SL alone (X3) were evaluated. No alterations were observed in the nutritional composition of the different formulations during the assessed storage period. The highest stability was observed for X1, which exhibited a decrease in red color intensity, while X2 and X3 exhibited increasing lightness. The highest scores for flavor, color, taste, overall acceptance and purchase intent were obtained for X1. These results reveal the potential of MP for use in French salad dressing as an emulsifier/stabilizer agent.

1. Introduction

Every year, the brewery industry discards tons of brewer's yeast used in industrial fermentation processes for the production of beer. This by-product could be destined for animal feed, however is generally discarded without reuse. In recent years, studies of the separation of the yeast cell components, especially the constituents of the cell wall, have demonstrated the important biological and technological properties of β-glucans and mannoproteins (MP), which represent up to 20 g/100 g of the cell dry weight (Araujo et al., 2014; Ding, Wang, Xiong, Zhao, & Huang, 2013).

MPs from brewer's yeast have interesting emulsifying and stabilizing properties that are chiefly related to the amphipathic structure of the MP molecule, in which hydrophilic polymers of mannose are joined to proteins (Cameron, Cooper, & Neufeld, 1988). Studies have also found that MP possesses good emulsifying and stabilizing effects *in vitro* when assayed at the various pH values and salt concentrations commonly applied by the food industry to formulate or preserve foods (Araujo et al., 2014). The advantages of using MP from yeast rather than synthetic emulsifiers stem from its biodegradable composition, lack of toxicity in humans and low-cost as a by-product. MP could be obtained from the yeast cell without previous treatment for removing residual products generated during the fermentation. Particularly, it is known that the residual ethanol resulting from beer fermentation processes does not affect the emulsification/stabilization ability of the extracted MP (Costa, Magnani, & Castro-Gomez, 2011). This polymer is easily extracted from the cell wall with a simple treatment with hot water followed by precipitation (Araujo et al., 2014), becoming viable the isolation in industrial scale. As a result, MP is an attractive product for industrial applications, including in the food industry (Torabizadeh, Shojaosadati, & Tehrani, 1996). However, its emulsifier properties must be assessed prior to the incorporation of MP in food formulations.

In recent years, salad dressing products have received growing attention in the food industry due to increased consumer demand for salads as a healthy food option. Salad dressings have a typical flavor and creaminess (Gomes, Gomes, Minim, & Andrade, 2008), which, when added to salads, can improve acceptance by consumers. Considering these aspects, the present study was conducted with the objectives of i) preparing a French salad dressing using MP from spent brewer's yeast as an emulsifying/stabilizing

* Corresponding author. Department of Food Engineering, Federal University of Paraíba, Campus I, 58051-900, João Pessoa, Paraíba, Brazil. Tel.: +55 83 3216 7576.
E-mail address: magnani2@gmail.com (M. Magnani).

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agent to totally or partially replace soy lecithin (LS), and ii) evaluating the effect of refrigerated storage on the nutritional composition, stability and sensory aspects of the prepared formulations.

2. Material and methods

2.1. Raw material and reagents

The ingredients used to prepare the salad dressing formulations (sunflower oil, sugar, salt, vinegar, skimmed milk and tomato extract) were purchased from a market in João Pessoa city, Paraíba, Brazil. Soy lecithin was kindly provided by the ROVAL company (João Pessoa, PB). The brewery slurry discarded after five fermentative processes (low fermentation) for beer production was provided by a brewery in Paraíba State.

2.2. MP extraction and characterization

The brewer's yeast slurry was sieved through a 0.297 mm mesh and diluted with distilled water (30 g/100 mL). After autolysis (55 °C; 24 h; 120 rpm) and hot water treatment (121 °C for 4 h), the material was centrifuged (4500 × *g* for 7 min at room temperature). To extract the MP, a supernatant of absolute ethanol was added (3 mL:1 mL), and the mixture was maintained at 4 °C for 18 h to precipitate the MP. The precipitate was obtained by centrifugation, washed with absolute ethanol (4500 × g, 5 min, 10 °C) and lyophilized (Araujo et al., 2014). MP obtained showed 39 g of mannose by 100 g of MP and 58 g of protein (of 58 kDa and 64 kDa) by 100 g of MP.

2.3. Elaboration and analysis of the French salad dressing

Three French salad dressing formulations were prepared according to the method of emulsification described by Gomes et al. (2008) using MP at concentrations of 0.8 g MP per 100 g (X1), 0.4 g MP and 0.4 g SL per 100 g (X2) and 0.8 g SL per 100 g (X3). The ingredients homogeneized to prepare the dressing consisted of sunflower oil (40 g/100 g), skimmed milk (8.5 g/100 g), vinegar (3 g/100 g), sugar (5.7 g/100 g), salt (1.5 g/100 g), tomato extract (8.5 g/100 g) and water (32 g/100 g). Samples of the dressing were used for physicochemical, technological, microbiological and sensory analyses. The dressings were analyzed after 1 day (following preparation) and 28 days of storage at 4 °C. Each day, three samples of the dressing from the same batch and experiment were collected. The samples were aseptically collected from different parts of the container for microbiological analysis. For stability analysis, 10 g of dressing was carefully collected. The remainder of the dressing was grated and immediately used for physical, chemical, microbiological and sensory analyses.

2.4. Determination of nutritional composition and pH

The nutritional composition of the dressing was determined in accordance with AOAC methods (2005) for moisture (925.09), fat (2000.18), protein (939.02), ash (930.30), and carbohydrate (920.124). The pH values were measured with a digital potentiometer.

2.5. Color analysis

A CR-300 colorimeter® (Minolta Co., Osaka, Japan) was used for instrumental color evaluation. The CIE Lab color scale ($L^*a^*b^*$) was used with a D^{65} illuminate (standard daylight) and a 10° angle. The L^*, a^* and b^* parameters were determined according to the International Commission on Illumination (CIE 1996). Using reference plates, the apparatus was calibrated in the reflectance mode with specular reflection excluded. A 10-mm quartz cuvette was used for the readings. The measurements were performed in triplicate (Mun et al., 2009).

2.6. Stability of the dressing formulations

The stability of the dressing formulations was evaluated after 1, 7, 14, 21 and 28 days of starage. In each period, the dressing formulations were heated at 80 °C for 30 min, cooled in running water (approximately 10 min) and centrifuged (3500 × *g*; 30 min; 25 °C). The emulsion stability was determined by comparing the measure of emulsion before and after heating, cooling and centrifugation according to a previously described procedure (Costa et al., 2011).

2.7. Sensory analyses of dressing

Sensory analyses of the dressing formulations were performed using effective acceptance and preference tests with 60 untrained tasters after 1 and 28 days of refrigerated storage. All tests were performed after obtaining approval from the Ethics Committee for Research Involving Human Beings (Process Number 10734712.8.0000.5188, Federal University of Paraíba, João Pessoa, Brazil) and after microbiological analyses to ensure the safety for human consumption according to the current legislation for microbiological criteria for foods (BRAZIL, 2001). For microbiological evaluation, counts of total and thermotolerant coliforms and coagulase-positive *Staphylococcus* were performed, and the presence of *Salmonella* spp. and *Listeria monocytogenes* were determined according to procedures described by the American Public Health Association (APHA, 2001, p. 676).

The sensory analyses were performed under controlled temperature and lighting conditions in individual booths. Each panelist received samples of dressing (50 mg) corresponding to the different formulations, which were served on a disposable white spoon coded with a random three-digit number. The samples were served simultaneously using a blind random sequence method immediately following removal from cold storage. The tasters were asked to eat a salty biscuit and drink water between the samples to avoid an aftertaste. For the acceptability of color, flavor, taste, texture and the overall assessment, a nine-point structured hedonic scale was used that ranged from one (strongly disliked) to nine (strongly liked). The intent to purchase was assessed using a five-point structured hedonic scale ranging from one (definitely would not purchase) to five (definitely would purchase) (Nikzade, Mazaheri, & Saadatmand-Tarzjan, 2012).

2.8. Statistical analysis

Statistical analyses were performed with descriptive statistics (mean and standard deviation) and inferential tests (ANOVA followed by Tukey's test) to determine significant differences ($P \leq 0.05$) between the treatments using the computer software Statistica 7.2.

3. Results and discussion

The French dressing made with MP alone (X1) showed no changes in pH during the 28 days of refrigerated storage ($p \leq 0.05$), with the pH measured as 6.0 (±0.02). For formulations X2 and X3, prepared with MP and SL or with SL alone, respectively, the pH values decreased during the assessed time ($p \leq 0.05$). The reduction of pH in emulsions has been associated with the occurrence of oil oxidation accompanied by the formation of hydroperoxides and with the hydrolysis of triglycerides accompanied by the formation

of fatty acids, both of which negatively affect the quality of the emulsion (Ghoush, Samhouri, Al-Holy, & Herald, 2008; Masmoudi, Le Dréau, Piccerelle, & Kister, 2005). In addition, pH is strongly related to the stabilization of emulsions prepared using protein emulsifiers (Guo & Mu, 2011; Worrasinchai, Suphantharika, Pinjai, & Jamnong, 2006). In this context, the consistency in pH values in X1 during the storage period could be an important factor responsible for the highest stability ($p \leq 0.05$) observed in this formulation when compared with X2 and X3 (Table 1). Similar behavior was previously reported for mayonnaise elaborated with increasing amounts of MP from the brewery yeast (0.6–1.0 g of MP/100 g of emulsion) during 28 days of refrigerated storage (Araujo et al., 2014).

Previous studies of the MP from the spent brewer's yeast *Saccharomyces uvarum*, the same yeast source evaluated in the present study, demonstrated good emulsifier/stabilizer properties over a large range of pH values and NaCl concentrations (Araujo et al., 2014; Melo et al., 2013). Most likely, these properties are a consequence of the amino acid composition of MP, which is dominated by hydrophobic amino acids, followed by neutral amino acids and lower amounts of hydrophilic amino acids (Melo et al., 2013). Considering the amino acid structure of MP, the highest stability observed for X1 and X2 (Table 1) could be due to the improved ability of MP to bind apolar and polar regions of different molecules compared with SL. According to Barriga, Cooper, Idziak, and Cameron (1999), the protein portion of MP from yeast is responsible for the emulsifying properties, acting in the interfacial tension, whereas the carbohydrate portion is responsible for the increase in the solubility of the polymer and for the stability of the formed emulsion.

Temperature is an important factor in emulsion stability during storage. Early studies reported that low temperatures could cause the crystallization of the two emulsion phases (oil and water), which could destabilize the emulsion (Palanuwech & Coupland, 2003). In the present study, low temperatures exerted a positive impact and increased the stability of the formulations. This effect may be due to the slow cooling employed. Still, the fatty acids composition of the sunflower oil, which contents high amounts (approx. 60%) of the polyunsaturated linoleic acid (C18:2) could also contribute for the stability and behavior observed for all dressing formulations during the refrigerated storage (Table 1). A previous study observed a negative correlation between the amounts of polyunsaturated fatty acids in the vegetable oils and the emulsion separation/desestabilization submitted to low temperatures (Magnusson, Rosén, & Nilsson, 2011).

The nutritional composition of the formulations did not change during the assessed time period ($p \leq 0.05$). Each 100 g of dressing contained, on average, 43.28 g moisture, 2.49 g ash, 2.94 g protein, 37.79 g fat and 13.48 g carbohydrate (Table 2), showing that the formulations displayed reduced fat content. This feature is important because salad dressings have been proposed for consumption with salads as part of a healthy diet (Gomes et al., 2008). Regarding the color analysis (Table 2) of X1 and X2, the two formulations containing MP, no differences ($p \leq 0.05$) were observed in terms of lightness (L^*) after 28 days of refrigerated storage. In contrast, X3 (made only with SL) displayed a decrease in this parameter during the studied period. The high values observed for L^* in the three formulations could be related to the regular formation of fat crystals as a result of the efficacy of the emulsifier/stabilizer (Sikimić, Popov-Raljić, Zlatković, & Lakić, 2010). The values of red intensity ($+a^*$) decreased in X1 and increased in X2 and X3 during the assessed time period. These variations could be related to the interaction between the MP and the carotenoids of the tomato extract used in the formulations. In studies of emulsions using yeast cell wall fractions as a fat substitute, whitish formulations have been observed (Volikakis, Biliaderis, Vamvakas, & Zerfiridis, 2004; Worrasinchai et al., 2006). The three formulations evaluated increased in yellow intensity ($+b^*$) during storage, most likely due to the pigments of the vegetable oil used in the formulations (Giovannucci, 1999).

For the assessed storage times, samples of dressing made with MP, SL, or with a mixture of both exhibited total and thermotolerant coliform counts <0.3 NMP/g, with an absence of coagulase-positive *Staphylococcus*, *Salmonella* spp. and *L. monocytogenes*. These results indicate that the French dressing samples assessed in this study possessed satisfactory microbiological quality according to current Brazilian legislation (BRAZIL, 2001).

The sensory analyses revealed that the formulation made only with MP (X1) received higher scores ($p \leq 0.05$) for all attributes (flavor, taste, color, texture and overall acceptance) compared with the scores obtained for X2 and X3 (Table 2). The formulations X2 and X3 showed similar scores for all attributes assessed, with no significant differences ($p \leq 0.05$). The average scores observed for overall acceptance were 7.88 for X1, 6.00 for X2 and 6.03 for X3, which correspond to "moderately liked", "slightly liked" and "slightly liked", respectively. The highest score (8.03) for the evaluated attributes was observed for the texture of X1, which corresponded to "liked". This result is interesting, as texture is one of the most important properties of an emulsion (Mun et al., 2009) and is related to the consistency and improved acceptance of salad dressings. In fact, higher purchase intention scores were obtained for X1 after 28 days of storage, with an average of 4.51, "possibly would purchase". These results suggest that the use of MP as an emulsifier/stabilizer in French salad dressings does not negatively affect the important sensory characteristics of the product, considering the superior results of the formulations with MP for acceptance and purchase intention when compared with formulations prepared with SL.

4. Conclusion

Our results demonstrated that French salad dressings made with MP from spent brewer's yeast exhibit good stability. In addition, the use of MP as an emulsifier in this type of sauce maintains the nutritional characteristics and improves the sensory acceptance and the stability of the product compared with formulations prepared with SL. These findings suggest that the MP from spent brewer's yeast slurries can be used as a bioemulsifier with potential

Table 1
Stability evaluation of French salad dressing formulations made with mannoprotein from *Saccharomyces uvarum* during 28 days of refrigerated storage.

Formulation*	1	7	14	21	28
X1	66.15 ± 0.67^{bA}	69.72 ± 0.65^{bA}	69.71 ± 0.36^{bA}	71.56 ± 0.66^{bA}	79.38 ± 0.33^{aA}
X2	35.68 ± 0.12^{bB}	55.41 ± 0.27^{aB}	55.59 ± 0.08^{aB}	56.63 ± 0.02^{aB}	58.02 ± 0.01^{aB}
X3	23.94 ± 0.22^{bC}	28.86 ± 0.15^{abC}	30.35 ± 0.37^{aC}	30.70 ± 0.15^{aC}	34.90 ± 0.02^{aC}

X1: 0.8 g of mannoprotein/100 g of dressing; X2: 0.4 g of mannoprotein/100 g + 0.4 g of soy lecithin/100 g of dressing; X3: 0.8 g of soy lecithin/100 g of dressing. $^{a-c}$For each trial, different superscript lowercase letters in an identical row denote differences ($P \leq 0.05$) between the mean values according to Tukey's test. $^{A-C}$For each trial, different superscript uppercase letters in an identical columns row denote differences ($p \leq 0.05$) between the mean values according to Tukey's test.

Table 2
Nutritional composition, color parameters and scores for the sensory evaluation of French salad dressing formulations made with mannoprotein from *Saccharomyces uvarum* during 28 days of refrigerated storage.

Formulation*	Days of storage	Chemical composition (g/100 g)					
		Moisture	Ash	Proteins	Lipids	Carbohydrate	
X1	1	44.44 ± 2.76[a]	2.51 ± 0.12[a]	2.86 ± 0.07[a]	37.42 ± 1.98[a]	12.77 ± 1.78[a]	
	28	42.11 ± 2.65[a]	2.46 ± 0.08[a]	3.02 ± 0.10[a]	38.16 ± 2.36[a]	14.19 ± 1.63[a]	
X2	1	42.93 ± 0.33[a]	2.53 ± 0.05[a]	2.83 ± 0.08[a]	36.09 ± 2.27[a]	15.63 ± 1.56[a]	
	28	42.90 ± 0.31[a]	2.46 ± 0.03[a]	2.79 ± 0.05[a]	34.22 ± 2.58[a]	17.64 ± 1.42[a]	
X3	1	40.86 ± 1.38[a]	2.49 ± 0.02[a]	2.89 ± 0.06[a]	30.11 ± 2.88[a]	23.65 ± 2.03[a]	
	28	42.46 ± 1.05[a]	2.48 ± 0.02[a]	2.67 ± 0.01[b]	30.84 ± 2.93[a]	21.54 ± 2.09[a]	
Formulation*	Days of storage	Color parameters					
		L^*	$+a^*$	$+b^*$			
X1	1	81.65 ± 0.25[a]	5.48 ± 0.32[a]	17.37 ± 0.37[b]			
	28	80.18 ± 0.12[a]	4,65 ± 0.33[b]	22.20 ± 0.33[a]			
X2	1	83.99 ± 0.11[a]	5.18 ± 0.02[b]	17.98 ± 0.33[b]			
	28	79.31 ± 0.68[a]	6,48 ± 0.27[a]	25.65 ± 0.01[a]			
X3	1	80.30 ± 0.66[a]	6.45 ± 0.66[b]	21.20 ± 0.26[b]			
	28	72.24 ± 0.22[b]	9.50 ± 0.13[a]	34.45 ± 0.02[a]			
Formulation*	Days of storage	Sensory atributtes					
		Flavor	Color	Taste	Texture	Overall acceptance	Purchase intention
X1	1	6.99 ± 1.04[a]	7.71 ± 0.89[a]	7.59 ± 1.28[a]	8.01 ± 0.91[a]	7.87 ± 0.92[a]	4.50 ± 0.64[a]
	28	7.02 ± 1.14[a]	7.74 ± 0.91[a]	7.60 ± 1.31[a]	8.03 ± 0.92[a]	7.88 ± 0.93[a]	4.51 ± 0.65[a]
X2	1	5.80 ± 1.21[b]	7.00 ± 1.72[b]	6.00 ± 2.05[b]	6.02 ± 1.99[b]	5.99 ± 1.64[b]	2.74 ± 1.07[b]
	28	5.90 ± 1.26[b]	7.00 ± 1.80[b]	6.01 ± 2.08[b]	6.04 ± 2.00[b]	6.00 ± 1.68[b]	2.75 ± 1.06[b]
X3	1	5.80 ± 1.53[b]	7.01 ± 1.68[b]	5.99 ± 2.12[b]	6.01 ± 2.02[b]	6.01 ± 1.70[b]	2.76 ± 1.05[b]
	28	5.82 ± 1.56[b]	7.03 ± 1.67[b]	6.00 ± 2.16[b]	6.03 ± 2.05[b]	6.03 ± 1.72[b]	2.77 ± 1.03[b]

X1: 0.8 g of mannoprotein/100 g of dressing; X2: 0.4 g of mannoprotein/100 g + 0.4 g of soy lecithin/100 g of dressing; X3: 0.8 g of soy lecithin/100 g of dressing. [a–b]For each trial, different superscript lowercase letters in an identical column denote differences ($p \leq 0.05$) between the mean values according to Tukey's test.

applications in the food industry as a substitute for synthetic agents and stabilizers, providing an alternative use for the most abundant by-product of breweries.

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